Part III
General Considerations of Skin Pigmentation

HISTORICAL BACKGROUND OF RESEARCH ON PIGMENTARY DISEASES OF THE SKIN

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The significant study of melanin pigmentation dates from relatively modern times. Eiselt, in 1861 (1), mentioned a few instances of probable melanoma from the literature of the 17th and 18th Centuries, with the statement that such reports are rare before the 19th Century, early in which Laennec (2) made the historical report on melanoma, which he called "la melanose". Eiselt quoted Highmore (1651), Bartholin (1677), Bonet (1679), Henrici and Nothnagel (1757) and others up to 1786 as having reported fatal black tumors with metastases and black fluid in the body, which were strongly suggestive of melanoma. He also stated that Walther reported in 1741 on the chemical behavior of black pigment. Robin (3) is credited with having been the first to use the term "melanin". He actually described the pigment in chromatophores of animals as "pigment melanique".

The advent of microscopic study made it inevitable that black pigment would excite the curiosity of early observers because its dark color furnished a contrast to normally non-pigmented cells. Most of the early microscopists studied only native unstained preparations with primitive microscopes. It is difficult for the modern worker with his research microscopes, fluorescent-, phase- and electron-microscopy, to appreciate the great credit which is due them for the excellent quality of their observations. In 1823 Heusinger examined the skin of Negroes, who, I think, have darker melanin than blond Caucasians. He identified a brown pigment in the stratum corneum, and irregular black little balls which are held together by "Malpighian mucus", beneath the superficial skin. He studied localized melanosis in freckles, liver spots, the skin of pregnant women, brown moles, in typhus and scurvy. He studied sections of melanoma and generalized pigmentation in scurvy, after poisoning, in yellow fever and other diseases. He demonstrated yellow, brown and black "stuff" in the mucosae (4).

Simon (5), in 1840, studied the skin of cadavers, and determined that the dark color of the areola is due to the presence of intracellular pigment. He teased the cells and likened the pigment granules contained therein to those found by Henle in Negro skin. In two large pigmented nevi, he localized most of the pigment in the rete malpighii, but there was a little in the dermis. Freckled skin also showed pigment in the rete.

In the study of skin of Caucasians, the anatomist Henle (6) wrote in 1843 that pigment is present at least temporarily in the nipple of women during pregnancy and lactation, in the labia majora and about the anus, and in men, in the skin of the penis and scrotum, with color about as intense as in Ethiopians. He also found pigment in freckles of blonds. He stated that a pigment layer is present between the epidermis and dermis over the entire body of Negroes. He localized the pigment cells between the corium and rete malpighii, but mixed with the latter, stating that they can be identified only by their content. Where the cutis is uneven, as, for example, the furrows between the papillae, they are concentrated in multi-layers.

Langerhans, in 1868 (7), by means of gold impregnation, identified nerves in the skin, and dendritic structures in the skin and hair matrix, which however, he did not identify with melanocytic function (see Fig. 1). Kromayer (1897) (8), stated that he had never seen a Langerhans cell attached to a nerve fiber. In 1871 Waldeyer may have been the first to describe isolated stellate pigment cells filled with light brown or golden

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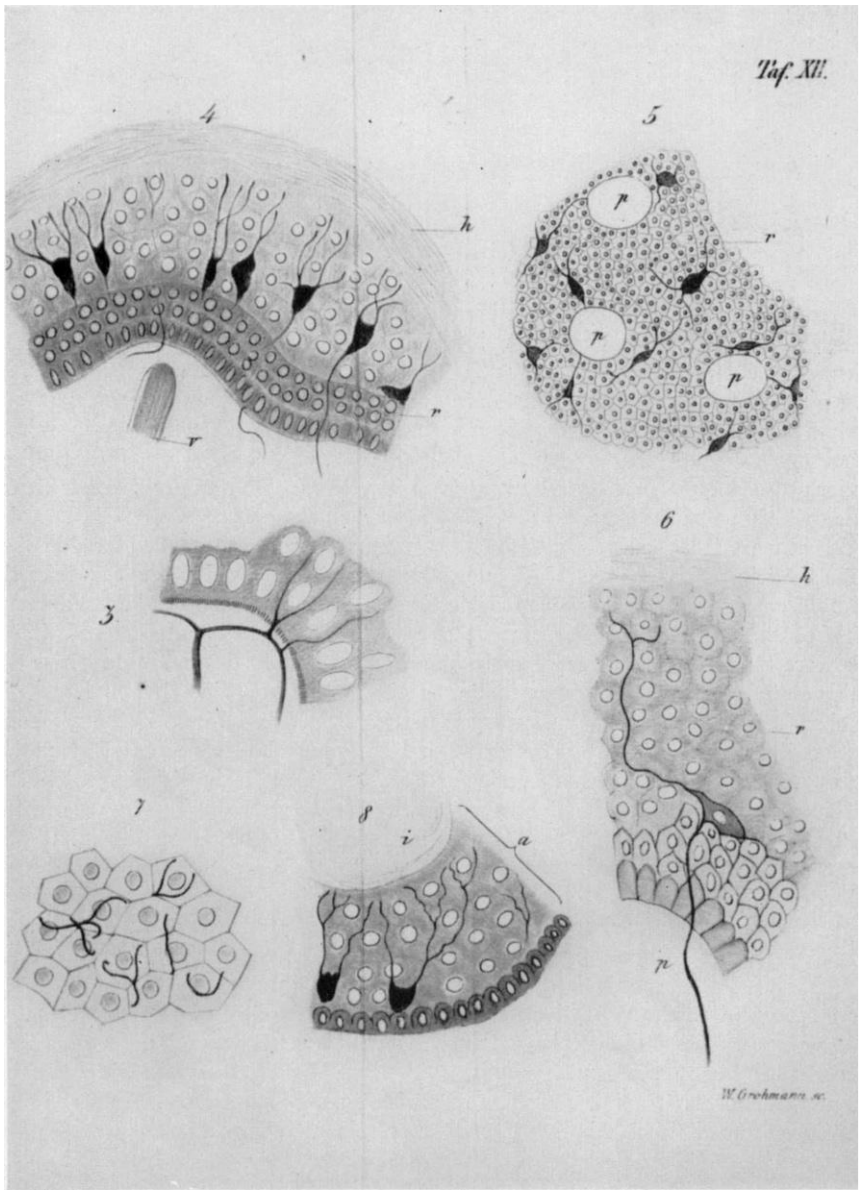


FIG. 1. Dendritic cells in epidermis. Hair matrix is shown in 8. (Silver impregnation.) From Langerhans, P. (7).

yellow granular pigment, in the connective tissue of the eyelids. They were located in the most superficial layers of the cutis (9). Riehl (1884) found melanocytes in the papillae of hairs (10). And in 1885, Aeby made the remarkable observation that dendritic cells give pigment to epithelial cells in caps on the distal pole of the nucleus (11).

Ehrmann in 1885, described pigment cells in the areola, skin of the genitalia, nape and hips in women (12a). He also published a small book (12b) on melanin pigmentation in human beings and animals. Bruno Bloch studied with Ehrmann in Vienna, and may have acquired much of his interest in pigmentation from this association.

Fetal material has certain advantages over adult skin for observation of melanocytes, because they become pigmented before the rest of epidermal tissue, and stand out in better con-

trast. Retterer (1887) was apparently the first to report observation of fetal pigment (13). In an eight cm. human fetus, pigment was found in some basal epidermal cells, but not in the dermis. In a 22 cm. (15 week) horse fetus, pigment granules were seen in the basal epidermal cells and in a 65 cm. horse fetus (after 29 weeks' gestation), some pigment was also seen in the hair. Meyersohn (14) studied the scalp of a five months old human fetus in 1889, seven year old child, six middle-aged brunets with beard or scalp hair, and sections from a white beard in

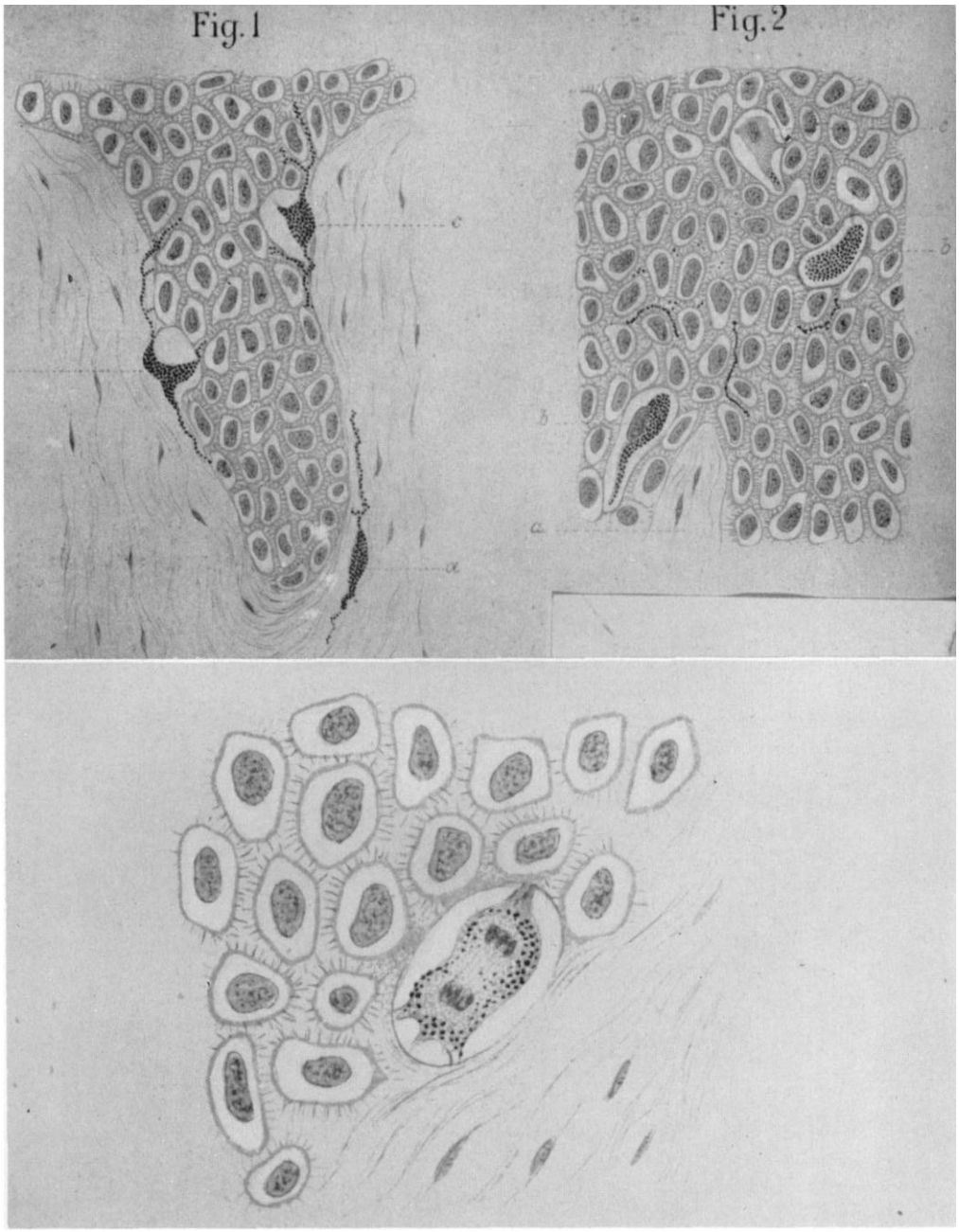


FIG. 2. (1 and 2). Melanocytes in epidermis. (3). Mitotic figure in melanocyte. From Bizzozero E. (18).

old age. He found dendritic cells in embryo and adult hair matrix, but none in white hair. He always found pigment in the dermis when the overlying epidermis was pigmented. He found branched cells in pigmented nevus, Addison's disease, and physiological pigmentation (pregnancy) only in the basal epidermis. He found dendritic cells in pigmented scleroderma. He also bleached tissue with chlorine water, and a mixture of hydrochloric acid and barium superperoxide, and found that nuclei still stained with carmine after the pigment granules could no longer be seen.

More recently, pigmentation of the Negro fetus was studied by Zimmermann and Cornbleet (15), who found melanocytes by the Bodian reduction silver stain and the dopa reaction in the third month of intrauterine life, after which time the dopa reaction became more strongly positive. The melanocytes were located in the intercellular epidermal spaces and formed an intricate pattern by their long processes. Becker Jr. and Zimmermann (16) studied the Negro fetus and found the first mature melanin granules in melanocytes of the eyelids, external auditory meatus and in specific areas of the oral mucosa in the third fetal month, about one month before they appear in the epidermis of the trunk regions. They were unable to stain melanoblasts before they reached the epidermis. The skin of the newborn Negro contained approximately 1030 dopa positive melanocytes per mm^2 (the same number as found by Szabo in adult skin). Gold impregnation of melanocytes in white fetuses was unsuccessful before the sixth month. Those revealed in the later stages were identical with gold impregnated melanocytes of the newborn Negro. Further studies by these workers (17) revealed that globular melanoblasts containing argentaffin granules may be identified in the Negro embryonic dermis during the tenth week of development by Masson's impregnation with ammoniated silver nitrate. The globular cells become oval and dendritic, at which time they are called melanocytes. These dermal melanocytes reach the epidermis and become epidermal melanocytes, where they join together and form a syncytium. In the scalp, sacral region, dorsum of the hand and foot, dermal melanocytes resemble those found in the ape. In the human being, the dermal melanocytes gradually become inactive. In the sacral region, they retain melanin long enough to form the mongolian spot. They are responsible for formation of blue nevi, and give origin to the rare melanoma arising in such nevi.

Bizzozero (18) in 1906, illustrated both Langerhans cells and melanocytes, as shown in Fig. 2. He also treated tissue by means of a silver impregnation method and introduced a new silver method which bears his name. He permitted two per cent silver nitrate to flow under the cover slip for two hours, then treated the section with cold saturated sodium thiosulphate solution for two minutes. Miescher studied this method, and believes that the dark substance so produced is a combination of melanin and silver, and does not constitute impregnation (19). The Bizzozero silver method and Bloch's dopa method constitute the best means of studying pigmented skin for determining the presence of melanin and melanocytic activity. The silver process darkens all melanin in both fresh and fixed tissues, but does not distinguish between melanocytes and melanophages, as does the dopa reaction.

The discovery of pigment in both the epidermis and dermis evoked more than the usual differences of opinion relative to its origin in one or the other situation, or both. The support for epidermal or dermal origin was about evenly divided. Caspary (20) considered the possibility of a combined origin, 1. from epidermis, and 2. transportation from dermis into the epidermis.

DISTRIBUTION OF MELANIN

Pigmentation of man is rather primitive when compared to that of some animals and birds. Becker Sr. demonstrated pigment in planarians (platyhelminthes) which resembles melanin (21). It is of interest to note that planarians are the lowest forms to possess a concentration of nerve

FIG. 3. Dopa reaction in epidermis (low power). From Bloch, B. and Ryhiner (23a).

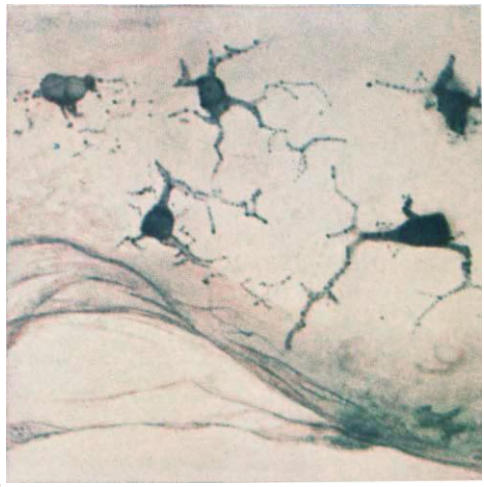
FIG. 4. Melanocytes in epidermis. (Dopa reaction, high power.) Primary and secondary dendrites may be seen. From Bloch, B. (23b).

FIG. 5. Tangential section showing syncytial arrangement of melanocytes. (Dopa reaction, high power.) From Bloch, B. (23b).

FIG. 6. Occupational melanosis. Dopa positive cells in epidermis and along hair follicle, showing syncytium. From Freund, E. (29).

Figure 3

Figure 4



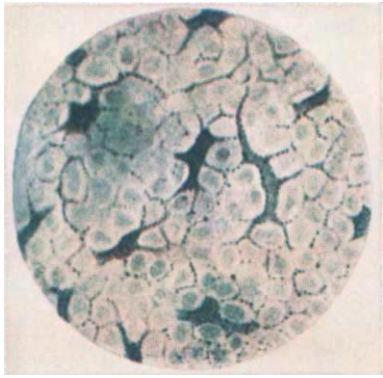


Figure 5

Figure 6

structures at the caudal extremity, which could be conceived as a primitive brain. Weidenreich (22) listed the pigment layers in lower forms as 1. ocular, 2. perineural, 3. perivascular, 4. pericoelomic, 5. dermal and 6. epidermal. In man, the surviving layers are the ocular, epidermal, and remnants of the perineural and dermal layers. His contribution is valuable in explaining the occurrence of evidently primary melanoma in the non-pigmented portions of the body on an atavistic basis.

FERMENT REACTIONS

In 1917, Bloch and Ryhiner (23) reported that frozen sections of fresh human skin, when placed in a 1:1000 aqueous solution of L-3,4,dihydroxyphenylalanine, called "DOPA" for short, at pH of 7.4, developed blackening of dendritic cells at the epidermo-dermal junction. He called such cells "melanoblasts" (see Figs. 3 and 4). Blackening also occurred in leucocytes from their contained polyphenolase, and in active cytochrome systems. The dopa solution darkened when exposed to air, and tissues left in the solution for a long period blackened throughout. These facts elicited opinions varying all the way to complete worthlessness. The reaction must be permitted to proceed only to an optimum point, and proves to be of great value if thus properly used.

The dopa-paraffin method of Becker Sr. *et al.* (24) enables better fixation of the tissues, and sectioning at different thicknesses. This procedure was valuable in evaluating the cells, both melanocytic and epidermal, and in establishing the individuality of the two types of cells. It had long been considered by several authors, including Rothman, that tyrosin was probably the mother substance of melanin, since dopa could not be demonstrated in the human skin. This concept was confirmed in the form of the tyrosin reaction, perfected by Fitzpatrick *et al.* (25), which corresponds closely to the dopa reaction, but is specific, while the dopa reaction is not. Raper (26) had shown that dopa was the first demonstrable intermediate in the tyrosin-tyrosinase reaction, and he identified several other intermediates leading to the formation of melanin. The tyrosin-tyrosinase reaction has been studied by many workers, including Lerner and Fitzpatrick, and Mason has contributed to the chemistry of melanin formation and to electron microscopic study of the melanin granules.

The arrangement of melanocytes in a syncytium at the epidermo-dermal junction was elaborated by Billingham (27) and Medawar. However, as early as 1911, Kreibich had stated that melanoblasts seem to build nets with each other (28). Bloch (23b) illustrated in 1917 a dopa reaction in a patient with lupus erythematosus, from whom the section had been cut tangentially to the cutaneous surface. He called attention to the syncytial form of the dendritic group, illustrated in Fig. 5. Freund, in 1926 illustrated a syncytium in melanosis from tar and oil (29). This has been reproduced in Fig. 6. Wieder illustrated a section that I had the pleasure of processing from a tangential cut along a hair follicle in sensitization melanosis, in which a syncytium is evident (30).

MELANOCYTE STIMULATING HORMONE

Zoologists have, for many years, been familiar with sudden change of color of certain animals. They also found that removal of the pituitary gland resulted in permanent lightening of the skin-color, which could be relieved by injection of pituitary extract. The increased pigmentation was found to be caused by scattering of pigment granules in melanophores in the skin. After the effect wore off, the pigment is again concentrated in the cell body and the color pales out.

In 1956, Lee and Lerner (31) announced the isolation of two melanocyte stimulating hormones (polypeptids) (alpha and beta MSH) from the hog pituitary gland. Injection of MSH in man produces rapid darkening of the skin and is alleged to cause formation of pigmented nevi (32).

ORIGIN OF MELANOCYTES

The origin of melanoblasts from the neural crest has been demonstrated by DuShane (33) for amphibians, by Dorris (34) for fowl, and by Rawles (35) for mice. The same experimental methods are not applicable to man, but the similarities of melanoblasts and melanocytes to nervous cells (staining by methylene blue, gold, silver), resistance to roentgen therapy on the part of melanoma, as are most malignant nervous tumors, and persistence of malignant melanoma cells in tissue culture in their own form with no reversion to epithelial cells, from which they were formerly alleged to have arisen, have convinced most observers that they are of nervous origin. Demonstration by Zimmermann and

Becker, Jr. (16) that melanoblasts migrate into the skin is of great significance.

SOLAR MELANOSIS

The most commonly observed hyperpigmentation is that caused by exposure to ultraviolet irradiation, from which very few persons escape. It is divided into two types, indirect and direct. The best studies on indirect melanosis are those of Edwards and Duntley (36), who, utilizing the Hardy spectrophotometer, identified pronounced hyperemia in two hours, with maximum intensity in 11 hours. Increase in melanin was apparent in two days, with maximum on the 19th day. At one month, melanosis started to diminish. At nine and one-half months, pigmentation had returned to the same level as before exposure. Miescher (1940) (37) stated that erythema is produced only by rays with wave length under 3200 Å, and Hamperl, Henschke and Schulze (38) reported in 1939 that exposure to such rays is followed by photochemical breaking up of nucleic acid in the nucleus, which, in turn, causes the erythema, in the form of an inflammatory reaction. Bachem (1929) placed the site of inflammation in the stratum germinativum or the dermis (39). Blum and Terus (1946) stated that pigment appears only after several days and varies in degree with intensity of the dosage of ultraviolet irradiation. Melanin first appears in dendritic cells in the basal layer of the epidermis (40).

Rothman (41) believes that the effect of ultraviolet irradiation is inactivation of inhibitors, chiefly sulfhydril compounds in the skin, which releases the tyrosinase so that pigment is formed. Lerner and Fitzpatrick (42) enumerate the factors in the melanocyte which influence pigmentation as: the presence of tyrosinase, oxygen, tyrosin, dopa, redox potential, sulfhydril groups, temperature and pH. Becker Jr. *et al.* (43) believe that after stimulation, melanin is retained in the dendritic cells, which is contrary to the previous idea that it was fed to the basal cells much as it is fed to hair and feathers in lower forms.

Direct pigmentation is caused, according to Miescher and Minder (44), by change of preformed oxygen-poor melanin into dark oxygen-rich melanin, which then migrates outwards. According to Henschke and Schulze, the wave length of ultraviolet responsible for direct pigmentation ranges from 3000 Å to 4300 Å (45). The action is rapid, and, according to Blum and Terus, begins immediately on exposure (40).

PHOTOSENSITIZATION

In 1897, White reported occurrence of what he called "dermatitis venenata" following the cleaning of parsnips (46). In the same year Stowers reported acute dermatitis in a man, aged 18, due to contact with young shoots (while gardening) of the cow parsnip (47). Both White and Stowers evidently attributed the eruption to some irritant, did not appreciate that they were caused by photosensitization and did not mention post-inflammatory melanosis. From these earliest reports to reports of the same dermatitis from the same cause by Starck (48) and Belisario (49) the mechanism of photosensitization has slowly been appreciated. The earliest recognition of cutaneous photosensitization seems to have been by Lewin in 1913, who reported the effect of sunshine on the skins of 103 workers in a cable factory where they were exposed to crude coal tar pitch. He verified the photosensitization by patch tests to tar products plus irradiation with a carbon arc lamp, using sunshine carbons (50). In 1916, Freund noted melanosis after the rubbing in of both *eau de cologne* and oil of bergamot, followed by exposure to the sun (51). Incidentally, he stated that a patch of vitiligo in the treated field did not repigment. In 1921, Legge reported stinging sensation, blistering and sanguineous oozing in pickers and packers of California figs, without mention of exposure to the sun or subsequent hyperpigmentation (52).

Guillaume, in 1927, made an important contribution when he showed that photosensitization to dyes (eosin, erythrosine, methyl violet and acridine) occurred only after scarification of the horny layer had permitted entry of the dyes into the prickle cells. If photosensitizing material did not penetrate the stratum corneum, it only acted as a protective filter (53). This fact explains the several reports by other authors of negative reaction to oil of bergamot, followed by irradiation.

Zurhelle, in 1928, stated that photosensitization dermatitis is more intense in blonds and melanosis is more pronounced in brunets (54). This fact accounts for the patients who have not noticed the dermatitis, but did develop rather pronounced melanosis.

Kuske experimented with fresh plant juices in 1940 and determined that all photosensitizing plants contain a related sensitizing substance, a furocumarin (55). It is in such a substance (methoxsalen) that we are currently interested.

Sams in 1941 studied photosensitization to lime oil in Florida (56).

MELANOSIS OF PREGNANCY

One of the most common of the melanoses and one of the earliest to be studied experimentally was the so-called physiological melanosis of pregnancy. That the hyperpigmentation is hormonal is strongly suggested by Meirowsky's report in the *Jadassohn Handbuch der Haut- u. Geschlectsk.*, that both of siamese twins became pigmented when only one of them was pregnant.

Steinach transplanted ovaries to castrated male guinea pigs in 1912. He illustrated enlarged nipples and hyperpigmented areola (57). Lipschütz (58), in 1930, transplanted ovaries into the kidney of castrated male guinea pigs. If the areola and nipple were in a pigmented area, they became hyperpigmented, but not if they were in an albinic area. Bloch and Schrafl (59) in 1932, confirmed Lipschütz' work and obtained the same results by feeding or injecting folliculin or oestroglandol. Bloch and Guldberg (60) produced even more intense pigmentation by subcutaneous injection of pure crystalline follicular hormone. They produced mild chloasma uterinum by injection into a woman, aged 22. Fierz (61) produced the same result by applying oestron or stilbestrol locally to one nipple. Local pigmentation ensued, but the other nipple was not affected. This result was confirmed by Davis *et al.* (62).

Lerner *et al.* (32) believed that melanosis of pregnancy may be due to MSH and progesterone, because the latter seems to have a direct MSH-like effect on the melanocytes.

MELANOMA IN ANIMALS AND ITS EXPERIMENTAL PRODUCTION

Fawdington (63), who published the first clinical illustration of human melanoma in 1826, stated that melanoma also occurs in dogs, swine, cats, rabbits and mice, but especially in horses, particularly those which have been dark-coated, but become white or gray.

Two mouse melanomas that have been used in the study of the disease are the Harding and Passey (64) and the Cloudman S91 (65) tumors. The Harding and Passey melanoma was used in tissue culture experiments by Grand *et al.* (66) and others. The Cloudman S91 has been especially useful as a source of tyrosinase, and the tumor has been studied in both its pigmented and non-pigmented form (67). Gesard, in 1903, demonstrated both tyrosin and tyrosinase in melanotic tumors from horses, which are not considered very malignant (68).

Efforts to reproduce melanoma experimentally have been discouraging. In 1924 Lipschütz painted mice every third day for seven months, at which time some mice developed benign melanomas (69). Passey, in 1938, found a long incubation period of six years or over in 12 airedale dogs he painted with tar. Three of the animals died early in the experiment. Three tumors developed, one melanoma, one malignant melanoma and a black tumor of undetermined nature (70). Schürch, in 1939 painted a rabbit's ear for three and one-half years with benzpyrene and injected oestroglandol for six months. A melanoma resulted that metastasized in lymph nodes, lungs, liver, spleen and kidney. The author stated that this was the first induced melanoma that has metastasized (71).

Hartwell and Stewart, in 1942, painted 5,9, 10 trimethyl-1,2 benzanthracene on the skin of mice (72). Pigmented foci appeared in 61 mice, but no definite neoplasms were found. My own efforts in painting gray house mice with tar and hairless mice, whose epidermis contained melanocytes, with benzpyrene produced tumors but no melanomas. Injection of the virus of the Rous chicken sarcoma into silkies, fowl which contain pigmented melanocytes in all tissue except epidermis and feathers, produced definite tumors but no melanomas.

Strong, in 1948, injected methylcholanthrene into parents of NHO descent of mice at 60 days age over many generations. Nineteen subcutaneous melanomas resulted, compared to six pigmented tumors in a large control group (73). Mulligan later reported 36 spontaneous melanomas in 31 dogs, 17 of which were non-cancerous. The other 19 were anaplastic, invasive and metastasized (74).

PIGMENTED NEVI AND MELANOMA

A voluminous literature has resulted from contributions to these subjects. There is still some

confusion from various differences of opinion. However, establishment of the melanocyte as of nervous origin has added greatly toward a simplification of this important problem.

ETIOLOGY OF VITILIGO

Many etiologic factors have been presented for vitiligo, including trophoneurotic, hormonal, infectious, toxic, medicamentous and inflammatory. My own concept is that it is a functional disorder, and must be treated as such, to avoid relapse. I believe that the uniformly high relapse rate, regardless of the type of treatment used, results from failure to relieve the patient's exhaustion. A distinct advantage of the use of methoxsalen is the fact that it is taken from a bottle. It is much easier to obtain a patient's cooperation if something is given to him, rather than just an outline of a regimen. Another important point is that all patches should be exposed to ultraviolet irradiation. I have seen vitiligo in a child which failed to respond only on the bathing trunk area, which was protected from the sun. Bloch (75) showed that vitiliginous patches give a negative dopa reaction, indicating failure of the ferment to function.

Jarret and Szabo have divided vitiligo into three groups, depending on the dopa reaction (76). If it is entirely negative, the vitiligo is classed as "Absolute". If the number of dopa positive melanocytes is unaltered, but the tyrosinase activity is reduced, it is called "Relative I". If the number of dopa positive cells is reduced and the remainder are large and have very long dendritic processes, it is called "Relative II".

TREATMENT OF VITILIGO BY ULTRAVIOLET IRRADIATION

The first record of therapy of vitiligo by ultraviolet irradiation was by D. W. Montgomery in 1904, who used a Finsen lamp on a Mexican boy, aged 19, who had had vitiligo since the age of five or six years (77). Some of his relatives were said to have had vitiligo. The boy was given nine exposures of ten minutes each. The areas became red as though sunburned. Some of the more recently depigmented plaques showed immediate improvement. In four months, the face was repigmented and the spots on the hands had nearly all disappeared. In 1907, Buschke reported failure with Finsen lamp and carbon arc lamp, but produced severe reaction with mercury vapor lamp. Pigment returned in from 13 days to three or four weeks. After one month's treatment, pigment returned about the hair follicles, and spread out to meet other islands (78). Stein verified this result, and also used heat, solid carbon dioxide and trauma with good results (79).

The first use of a photosensitizer was by Uhlmann in 1927 (80a) who applied oil of bergamot and exposed the areas to the sun's rays or Kromayer lamp. Pigment appeared and remained during nine months' observation. He later reported that he had treated over 50 patients by this method with good results (80b). Other photosensitizers that have been applied to the skin have been eosin, cod-liver-oil, paste of seed of *Psoralia corylifolia*, and furocumarins from *Ammi majus*. Internal medication has been injections of oil of *Psoralia corylifolia*, gold, trypaflavin, acridin, and oral administration of material from *Ammi majus*, all associated with local administration of ultraviolet irradiation.

In closing, please be reminded that, in 50 years or so, a group similar to ours will be discussing the treatment of vitiligo and evaluating the statements that we have made. Let us hope that they will be as gracious toward us as we have tried to be toward our predecessors in research on pigmentary and depigmentary disorders.

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